Molecular Methodes

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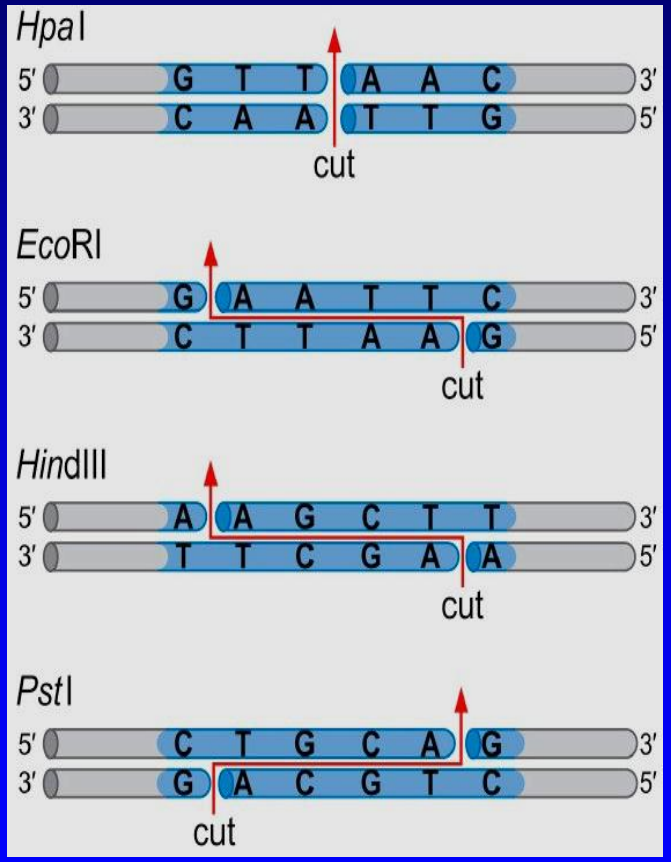
Restriction Endonucleases



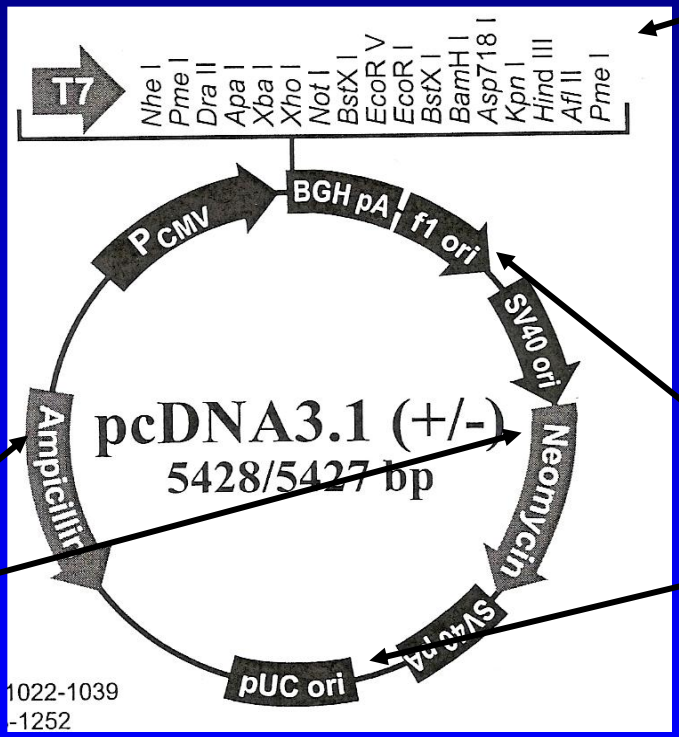
Blunt end

Sticky ends

Cloning DNA into Plasmid Vectors

When working with a defined piece of DNA it is oftentimes necessary to clone the gene (or insert the gene) into a second DNA molecule that allows the propagation of the DNA in bacteria along with a simple transfer to and from bacteria and mammalian cells. These closed circular DNA molecules are called **plasmid vectors**. Plasmid vectors are autonomously replicating pieces of DNA and must have the following characteristics:

Multiple Cloning Site: This is a region that contains restriction sites that are unique within the vector. This allows DNA fragments to be inserted at a defined point within the vector.



Selectable Markers:

These allow for the selection of either bacteria (Ampicillin) or eukaryotes (Neomycin) that have received and contain the plasmid vector.

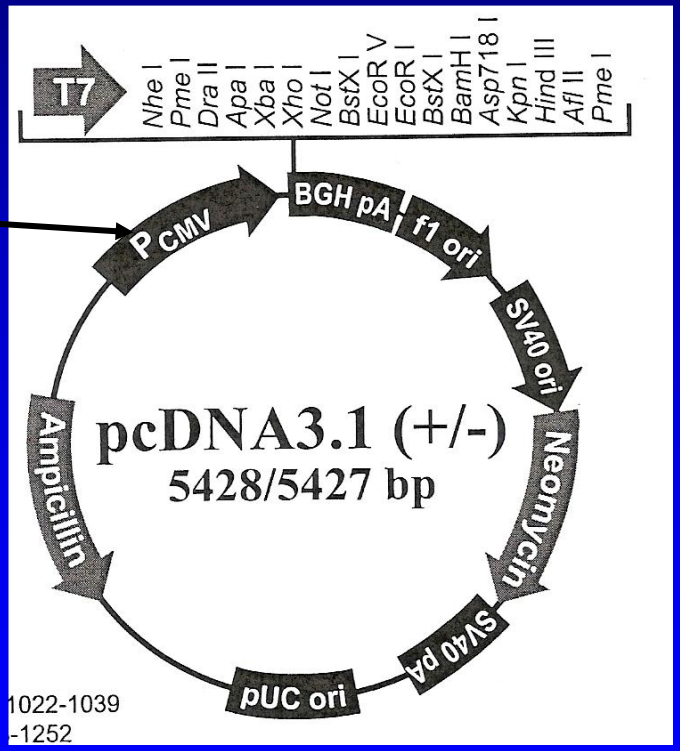
Origins of replication: These allow the vectors to replicate independent of the host. (pUC ori - bacteria; SV40 ori - eukaryotes)

Cloning DNA into Plasmid Vectors

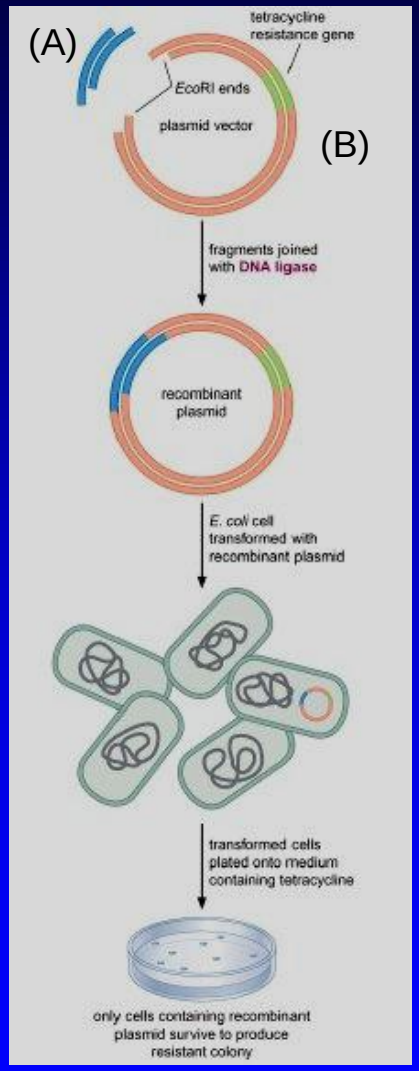
Some plasmid vectors can also be **expression vectors**. These vectors (like the one listed below) contain an additional DNA element called a promoter. These promoter elements can be either from eukaryotic origin (allowing the expression of the desired protein in eukaryotes) or prokaryotic in origin (allowing the expression of the desired protein in prokaryotes).

Eukaryotic Promoter:

The cytomegalovirus (CMV) promoter allows the constitutive (or constantly being expressed) expression of the gene that has been cloned into the MCS immediately downstream of it.



Cloning DNA into Plasmid Vectors



- To clone a piece of DNA into a plasmid vector:
  - Vector (A), which contains the DNA fragment of interest, is digested with restriction enzymes that will release the entire fragment from the vector.
  - The released DNA fragment is separated from the parent vector by agarose gel electrophoresis with subsequent purification of the fragment from the gel.
  - The target vector (B), is also digested with the identical restriction enzymes, which generates a linearized backbone vector with complementary DNA overhangs, and gel purified.
  - The DNA fragment (A) is then joined with the target vector (B) using another enzyme called **DNA Ligase**. [This enzyme creates a covalent bond between the insert DNA and the backbone vector DNA at the overhangs where the two pieces of DNA are complementary].
  - The ligated DNA is transformed into bacteria (that is, chemical or electrical means are used to transfer the ligated DNA into bacteria) and grown on plates that contain ampicillin.
  - Because of the ampicillin resistance gene on the vector, only those bacteria that contain the ligated vector will be able to grow in the presence of the antibiotic.
  - Pick some of the bacterial colonies, isolate the DNA, and confirm the presence of the correctly formed clone by additional restriction digests.

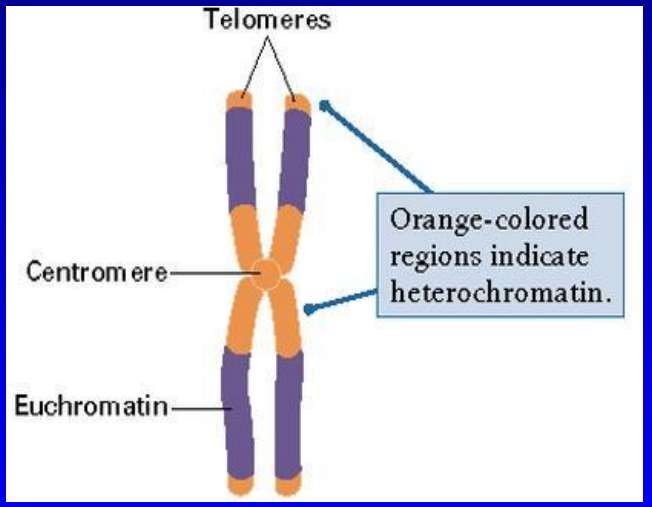
  NOTE: in some cases, only one restriction enzyme is used generating the identical ends on the 5'- and 3'-ends of the insert fragment (**bidirectional cloning**). In these cases it is necessary to use another restriction enzyme to confirm the correct orientation of your cloning.

Cytogenetics

Types of chromatin :

1) **Euchromatin** is the less compact DNA form, and contains genes that are frequently expressed by the cell.

2) **Heterochromatin**, is the more compact form, and contains DNA that are infrequently transcribed.

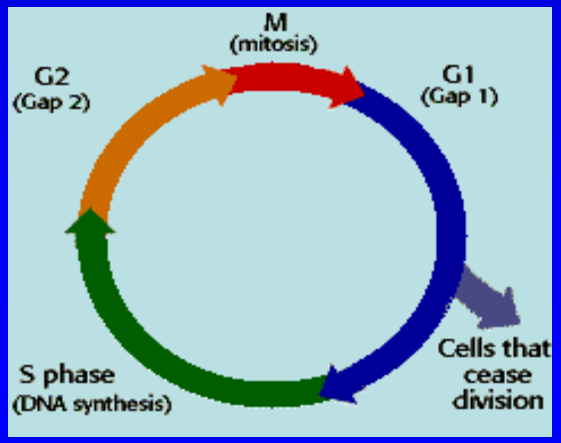


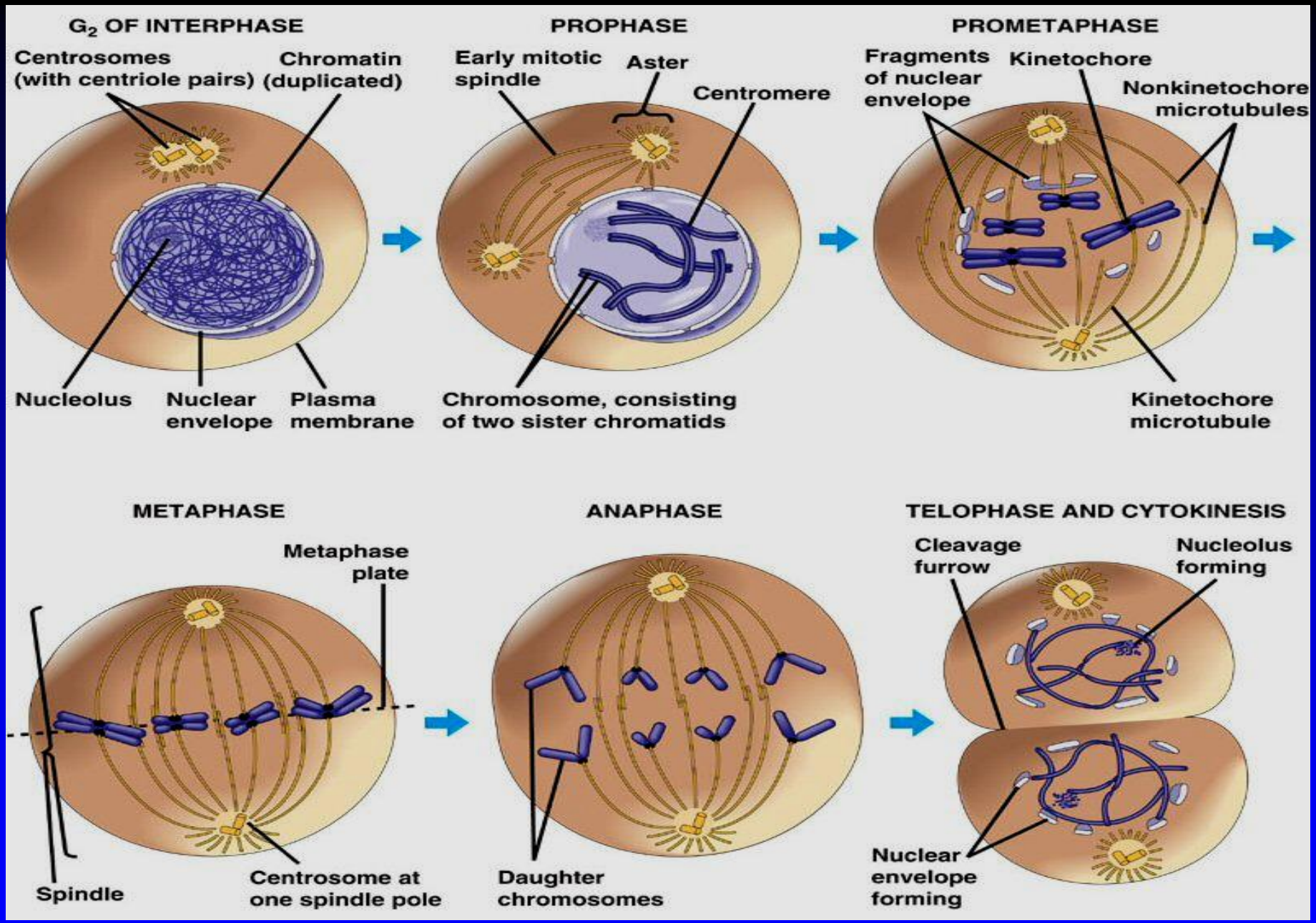
This structure is further categorized into facultative heterochromatin, consisting of genes that are organized as heterochromatin only in certain cell types or at certain stages of development, and constitutive heterochromatin that consists of chromosome structural components such as telomeres and centromeres.

Cell division (mitotsis)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components.

Mitosis and cytokinesis together define the mitotic (M) phase of the cell cycle—the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.





Phases of cell mitotic division

Chromosomal abnormalities

1) Numerical Disorders (Aneuploidy)

a) **Monosomy**; an individual is missing either a chromosome from a pair .

Ex: Turner Syndrome, where the individual is born with only one sex chromosome, an X.

b) Trisomy ; has more than two chromosomes of a pair.

Ex: Down Syndrome, also known as Trisomy 21 (an individual with three copies of chromosome 21, rather than two).

2) Structural abnormalities

Deletions: Ex: cri du chat (deletion of 5q).

Translocations: When a portion of one chromosome is transferred to another chromosome.

Inversions: the genetic material is inverted.

Rings: A portion of a chromosome has broken off and formed a circle or ring.

Chromosome instability syndromes They often lead to an increased tendency to develop certain types of malignancies.

Karyotyping

Types of banding

Cytogenetics employs several techniques to visualize different aspects of chromosomes:

1)G-banding; It yields a series of lightly and darkly stained bands - the dark regions tend to be heterochromatic. The light regions tend to be euchromatic.

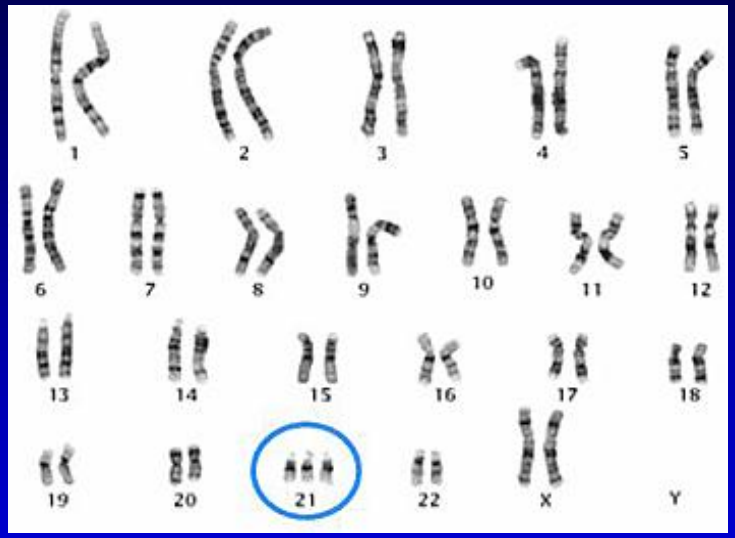
2) R-banding; is the reverse of G-banding (the R stands for "reverse"). The dark regions are euchromatic and the bright regions are heterochromatic.

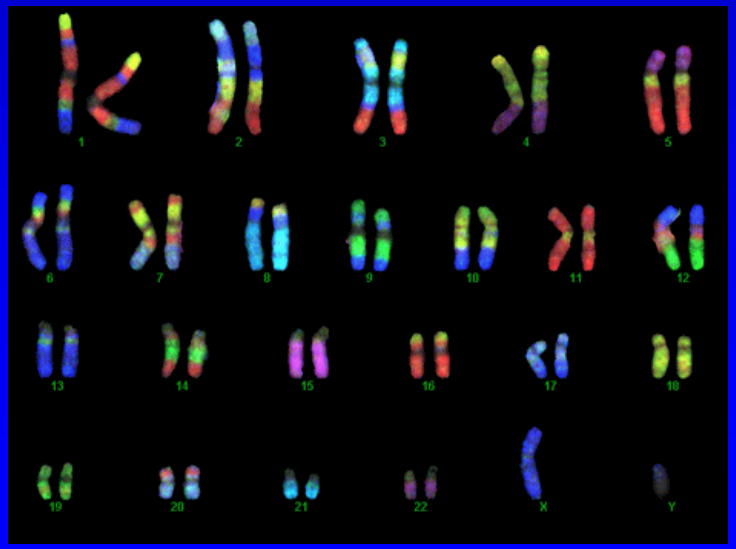
3)C-banding: Giemsa binds to constitutive heterochromatin, so it stains centromeres.

4)T-banding; visualize telomeres.

5)Q-banding; is a fluorescent pattern obtained using quinacrine for staining. The pattern of bands is very similar to that seen in G-banding.

Karyotyping



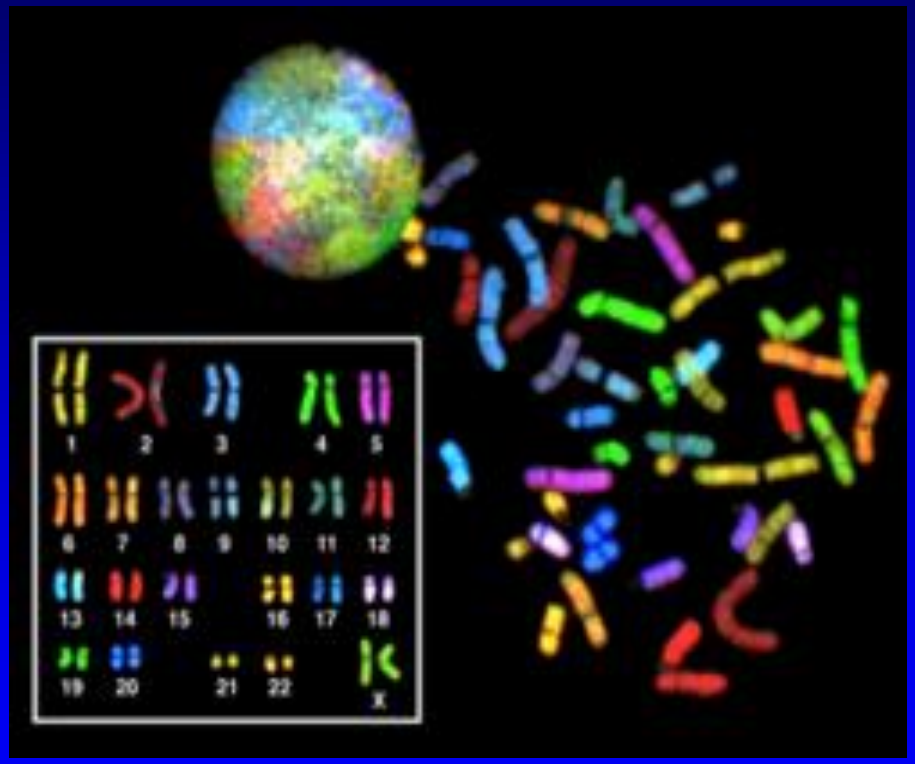


Classic karyotyping

In the classic karyotype, a dye, often Giemsa (G-banding), less frequently Quinacrine, is used to stain bands on the chromosomes (giemsa is specific for the phosphate groups of DNA; quinacrine binds to the adenine-thymine-rich regions). Each chromosome has a characteristic banding pattern that helps to identify them; both chromosomes in a pair will have the same banding pattern.

Karyotypes are arranged with the short arm of the chromosome on top, and the long arm on the bottom. Some karyotypes call the short and long arms p and q, respectively.

Karyotyping



Spectral karyotype (SKY technique)

Spectral karyotyping is a molecular cytogenetic technique used to simultaneously visualize all the pairs of chromosomes in an organism in different colors. Fluorescently labeled probes for each chromosome are made by labeling chromosome-specific DNA with different fluorophores (the fluorescence probes).

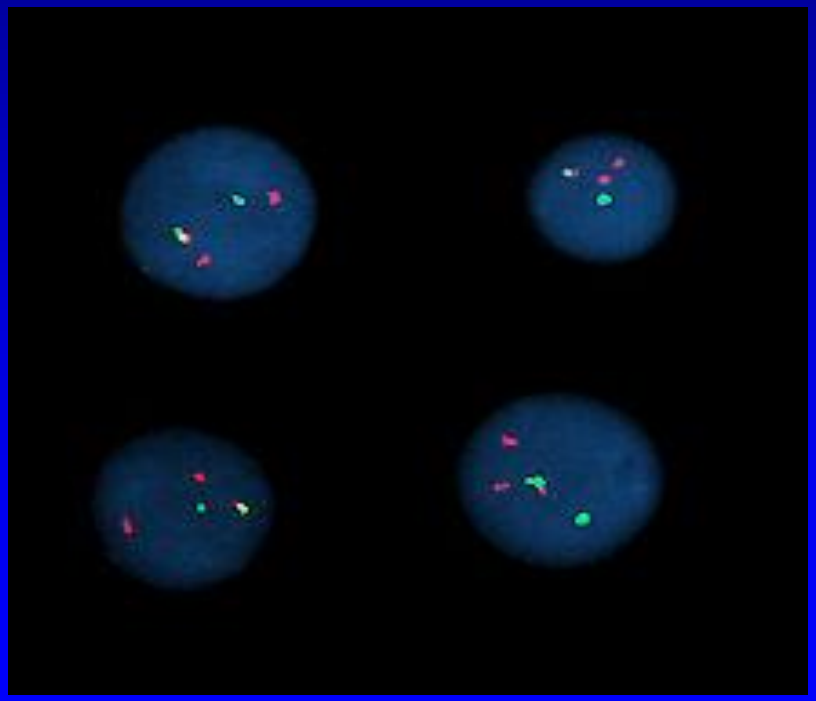
Karyotyping

Digital karyotyping

Digital karyotyping is a technique used to quantify the DNA copy number on a genomic scale (for detection of a specific gene deletion or duplication). Short sequences of DNA from specific loci all over the genome are isolated and enumerated. This method is also known as virtual karyotyping.

Fluorescent in situ hybridization (FISH) karyotyping

Fluorescent in situ hybridization refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations (for detection of a specific gene translocation).



Interphase cells positive for a t(9;22) rearrangement

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